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Research Article

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Synthesis and antitumor properties of bis-indole derivatives

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ABSTRACT

A series of bis-indole derivatives were designed and synthesized about 10-20% overall yields. Four newly synthesized compounds have not been reported before. Their structures were characterized by ^1H NMR. All the synthesized compounds were evaluated for inhibition of cell proliferation of human liver cancer (HepG2), human colon cancer (HT-29) and human leukemia (K562) cell lines activity. The bis-indole showed the inhibition of K562 with IC_{50} values of 56.19-58.55 μM, respectively.

Keywords: bis-indole derivatives, anti-tumor activity, MTT

INTRODUCTION

Indole belongs to the indole alkaloids that are widely existed in nature resource. Indole and its derivatives have been reported to exhibit a range of biological and pharmacological activities [1-4], such as anti-tumor, anti-bacterial, anti-hypertensive and anti-Alzheimer's [5-7]. In recent years, indole and its derivatives have been widely concerned by their special character and structure. Bis(indole) alkaloids, consisting of two indole moieties connected to each other via heterocyclic units, have been particularly abundant within sponges[8].

Many researches have been focused on the synthesis and biological evaluation of the bis-indole derivatives. Bis-indole derivatives as an important class of heterocyclic compounds have become known. And they are intermediate products with biological activity in research and development and pharmaceutical industries. They can not only increase the natural metabolism of hormones in the body, but also prevent the cancer effectively. In this paper, we would like to report the design, synthesis and biological evaluation of bis-indole derivatives. The general procedures for the preparation of novel bis-indole derivatives 5a-d are outlined in scheme 1, herein we would like to report the synthesis and antitumor activity of a series of bis-indole derivatives against three human cancer cell lines, including human leukemia K562, human liver cancer HepG2 and human colon cancer HT-29.

EXPERIMENTAL SECTION

2.1 Materials and measurements

Used in this article, all reagents and solvents were of analytical grade. The reaction temperature control uses the oil bath temperature modulator. Thin layer chromatography (TLC) with silica gel 60 GF254. Merck precoated plates (0.25 mm) was visualized using UV. 0.1 for flash chromatography on silica gel (particle size 100-200 mesh). ^1H spectra were recorded in DMSO on Bruker AM-400 NMR spectrometers using tetramethylsilane (TMS) as an internal standard. The chemical shifts are reported in δ (ppm) relative to tetramethylsilane as internal standard.

2.2 Synthesis route of Bis-indole derivatives

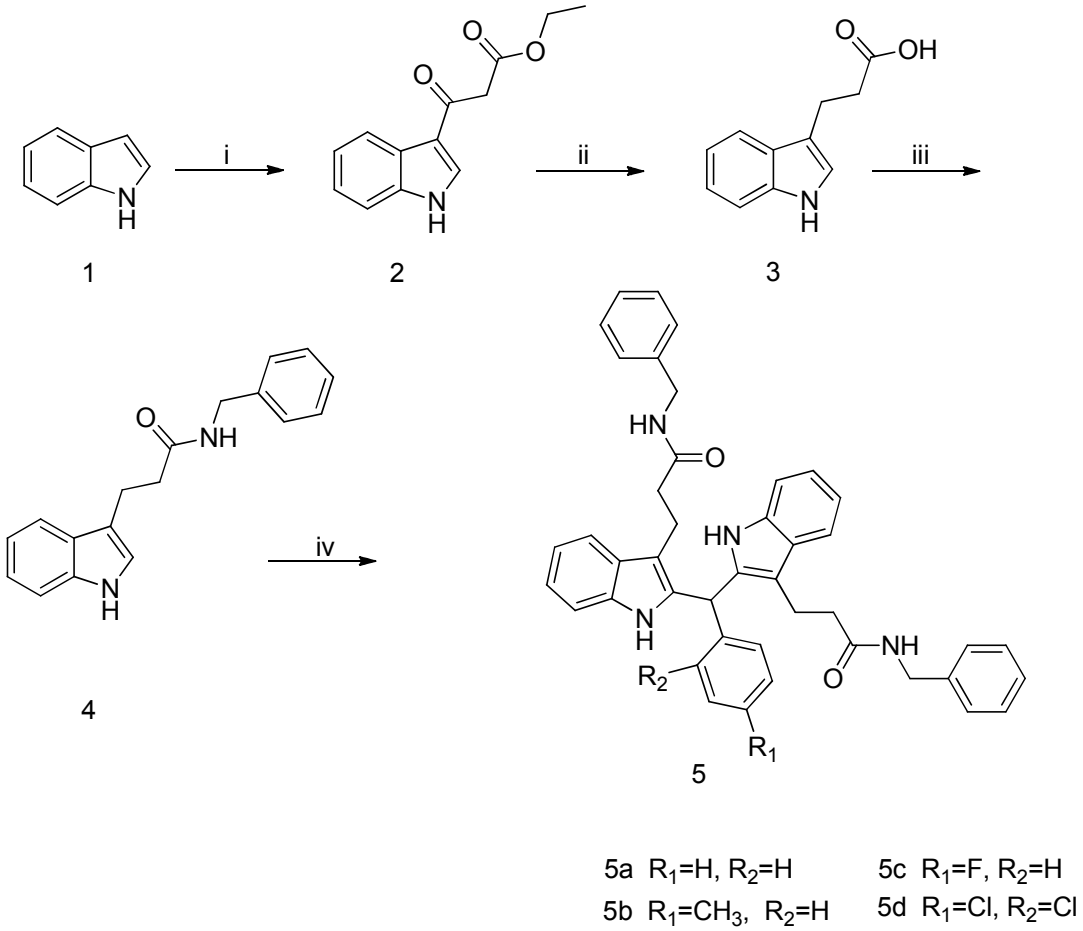


Fig 1: synthesis route of Bis-indole derivatives

Reagents and conditions: (i) $AlCl_3$, CH_2Cl_2, Ethyl oxalyl monochloride, 35 ℃, 4 h; (ii) $NH_2NH_2{\cdot}H_2O$, Ethanol, KOH, 65 ℃, 2 h; (iii) Et_3N, THF, Benzylamine, r.t., 1.5 h; (iv) Bismuth(III) trifluoromethanesulfonate, Benzaldehyde, CH_3CN, 35 ℃, 3 h.

2.2.1 3,3'-(2,2'-(phenylmethylene)bis(1H-indole-3,2-diyl))bis(N-benzylpropanamide) (5a)

To a solution of indole (13.80 g, 0.10 mol) in DCM was added $AlCl_3$ (14.70 g, 0.11 mol) and Ethyl oxalyl monochloride (15.00 g, 0.11 mol) below 0 ℃. After complete addition, the reaction was stirred under reflux for 6 h. The reaction mixture was quenched with H_2O (100 mL). The solids were collected and filtrated to afford ethyl 3-(1H-indol-3-yl)-3-oxopropanoate (20.81 g, yield 87%).

To a solution of ethyl 3-(1H-indol-3-yl)-3-oxopropanoate (17.90 g, 75 mmol) in ethanol was added 85% $NH_2NH_2{\cdot}H_2O$ (22.10 g, 37.50 mmol) and KOH (12.60 g, 22.50 mmol) below 20 ℃. After complete addition, the reaction was stirred under reflux for 4 h. The reaction mixture was poured into 6 M HCl and the precipitate collected and washed with water. The product was a white solid (10.80 g, 73%).

To a solution of 3-(1H-indol-3-yl)propanoic acid (0.50 g, 2.64 mmol) in dry THF was added triethylamine (1.10 mL, 7.93 mmol), chloroformates (0.26 mL, 2.77 mmol) and benzylamine (0.58 ml，5.29 mmol) below 0℃. After complete addition, the reaction was stirred at 35℃ for 2 h. The reaction mixture was poured into 50 mL of ice water and extracted the mixture with dichloromethane three times. The combined organic layers were dried over $MgSO_4$, filtered and the ether removed. Further purification was performed with silica gel chromatography using PE:EA (15:1-5:1) as the eluent to afford N-benzyl-3-(1H-indol-3-yl)propanamide (0.57 g, 78%).[9,10]

To a solution of N-benzyl-3-(1H-indol-3-yl)propanamide (0.10 g, 0.38 mmol) in CH_3CN was added Bismuth(III) trifluoromethanesulfonate (94.30 mg, 0.14 mmol) and benzaldehyde (88.60 mg，0.84 mmol) below 0℃. After complete addition, the reaction was stirred at 35℃ for 3 h. The reaction mixture was poured into 50 mL of ice water and extracted the mixture with dichloromethane three times. The combined organic layers were dried over $MgSO_4$, filtered and the ether removed. Further purification was performed with silica gel chromatography using PE:EA

(10:1-4:1) as the eluent to afford the title compound **5a** (0.14 g, 60%).

2.2.2 3,3'-(2,2'-(p-tolylmethylene)bis(1H-indole-3,2-diyl))bis(N-benzylpropanamide) (5b)
To a solution of N-benzyl-3-(1H-indol-3-yl)propanamide (0.10 g, 0.38 mmol) in CH_3CN was added Bismuth(III) trifluoromethanesulfonate (94.30 mg, 0.14 mmol) and p-Tolualdehyde (0.10 g, 0.84 mmol) below 0℃. After complete addition, the reaction was stirred at 35℃ for 3 h. The reaction mixture was poured into 50 mL of ice water and extracted the mixture with dichloromethane three times. The combined organic layers were dried over $MgSO_4$, filtered and the ether removed. Further purification was performed with silica gel chromatography using PE:EA (10:1-3:1) as the eluent to afford the title compound **5b** (0.12 g, 52%).

2.2.3 3,3'-(2,2'-((4-fluorophenyl)methylene)bis(1H-indole-3,2-diyl))bis(N-benzylpropanamide) (5c)
To a solution of N-benzyl-3-(1H-indol-3-yl)propanamide (0.10 g, 0.38 mmol) in CH_3CN was added Bismuth(III) trifluoromethanesulfonate (94.30 mg, 0.14 mmol) and 4-Fluorobenzaldehyde (0.10g, 0.84 mmol) below 0℃. After complete addition, the reaction was stirred at 35℃ for 3 h. The reaction mixture was poured into 50 mL of ice water and extracted the mixture with dichloromethane three times. The combined organic layers were dried over $MgSO_4$, filtered and the ether removed. Further purification was performed with silica gel chromatography using PE:EA (10:1-5:1) as the eluent to afford the title compound **5c** (0.10 g, 43%).

2.2.4 3,3'-(2,2'-((2,4-dichlorophenyl)methylene)bis(1H-indole-3,2-diyl))bis(N-benzylpropanamide) (5d)
To a solution of N-benzyl-3-(1H-indol-3-yl)propanamide (0.10 g, 0.38 mmol) in CH_3CN was added Bismuth(III) trifluoromethanesulfonate (94.30 mg, 0.14 mmol) and 2,4-dichlorobenzaldehyde (1.47 g, 0.84 mmol) below 0℃. After complete addition, the reaction was stirred at 35℃ for 3 h. The reaction mixture was poured into 50 mL of ice water and extracted the mixture with dichloromethane three times. The combined organic layers were dried over $MgSO_4$, filtered and the ether removed. Further purification was performed with silica gel chromatography using PE:EA (10:1-2:1) as the eluent to afford the title compound **5d** (0.15 g, 60%).

2.3 Biological assay.
The cell lines HepG2, HT-29 and K562 were plated in 96-well plates at a density of 5×10^3 cells per well and cultured at 37℃ in 5% CO_2 for 2 h (suspension cells) or 24 h (attached cell). Cells were treated with different concentrations of compounds and incubated at 37 °C for an additional 48 h. MTT assay was performed using Thermo microplate reader. The DMSO-treated controls were calculated as a cell viability value of 100%. The IC_{50} values were obtained by nonlinear regression using GraphPad Prism 4.0. IC_{50} measurements for each compound were done three times.

RESULTS AND DISCUSSION

3.1 Characterize isatin derivatives by ^{1}H NMR.
3.1.1 3,3'-(2,2'-(phenylmethylene)bis(1H-indole-3,2-diyl))bis(N-benzylpropanamide) (5a)
^{1}H NMR (400 MHz, DMSO): δ/ppm 2.43 (t, J=6.8 Hz, 4H), 2.90-2.98 (m, 2H), 3.02-3.09 (m, 2H), 4.19 (d, J=6.0 Hz, 4H), 6.22 (s, 1H), 6.94-7.05 (m, 6H), 7.13 (d, J=7.2 Hz, 4H), 7.17-7.32(m, 11H), 7.52(d, J=7.6Hz, 2H), 8.29(t, J=5.8Hz, 2H), 10.45 (s, 2H).

3.1.2 3,3'-(2,2'-(p-tolylmethylene)bis(1H-indole-3,2-diyl))bis(N-benzylpropanamide) (5b)
^{1}H NMR (400 MHz, DMSO): δ/ppm 2.26 (s, 3H),2.42 (t, J=6.8 Hz, 4H), 2.87-2.98 (m, 2H), 3.01-3.09 (m, 2H), 4.19 (d, J=6.0 Hz, 4H), 6.16 (s, 1H), 6.94-7.05 (m, 6H), 7.13 (d, J=7.2 Hz, 4H), 7.17-7.32 (m, 11H), 7.52 (d, J=7.6Hz, 2H), 8.27 (t, J=5.8Hz, 2H), 10.39 (s, 2H).

3.1.3 3,3'-(2,2'-((4-fluorophenyl)methylene)bis(1H-indole-3,2-diyl))bis(N-benzylpropanamide) (5c)
^{1}H NMR (400 MHz, DMSO): δ/ppm 2.43 (t, J=6.8 Hz, 4H), 2.90-2.98 (m, 2H), 3.02-3.09 (m, 2H), 4.19 (d, J=6.0 Hz, 4H), 6.41 (s, 1H), 6.94-7.13 (m, 12H), 7.17-7.26 (m, 6H), 7.31 (d, J=7.6 Hz, 2H), 7.52 (d, J=8.8 Hz, 2H), 8.29 (t, J=5.8Hz, 2H), 10.42 (s, 2H).

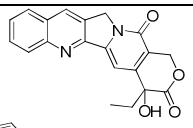
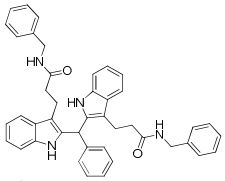
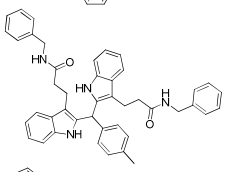
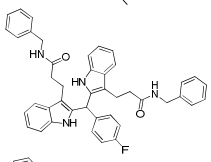
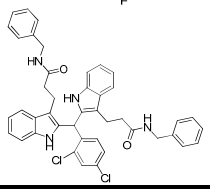
3.1.4 3,3'-(2,2'-((2,4-dichlorophenyl)methylene)bis(1H-indole-3,2-diyl))bis(N-benzylpropanamide) (5d)
^{1}H NMR (400 MHz, DMSO): δ/ppm 2.31-2.35 (t, J=14.4 Hz, 4H), 2.75-2.86 (m, 4H), 4.18 (d, J=6.0 Hz, 4H), 6.35 (s, 1H), 6.96-7.19 (m, 9H), 7.21-7.37 (m, 8H), 7.38-7.40 (d, J=8.0 Hz, 1H), 7.52-7.54 (d, J=8.0 Hz, 2H), 7.64 (s, 1H), 8.20-8.23 (t, J=5.6 Hz, 2H), 10.44 (s, 2H).

3.2 Anticancer activity assay.
The newly prepared bis-indole **5a-5d** were evaluated for their in vitro cytotoxic effects against human leukemia

K562, human liver cancer HepG2, and human colon cancer HT-29 cell lines by the standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay with Camptothecin (CPT) as the positive control. The preliminary results were summarized in Table 1.

Among all the screened compounds, **5a**, **5b**, **5c**, and **5d** showed weaker potency against human liver cancer HepG2, and human colon cancer HT-29 cell lines (Table 1). Compounds **5a**-**5d** exhibited moderate activities against human leukemia K562. These interesting results might be used to develop novel bis-indole for potential therapy human leukemia agents. Among, compounds **5a** and 5**c** exhibited better anticancer activity, with the IC_{50} values of 56.91 μM and 58.55 μM, against cancer cell lines K562. The data suggested that the presence of electron withdrawing groups on the phenyl ring be able to improve anticancer activity.

Table 1 Bis-indole derivatives for the inhibition of tumor cells

Compd.	Structure	IC_{50} (μM)		
		K562	HepG2	HT-29
CPT		0.007	0.05	0.2
5a		56.19	>100	>100
5b		>100	>100	>100
5c		58.55	>100	>100
5d		>100	>100	>100

CONCLUSION

A series of bis-indole derivatives have been designed and synthesized as potential cytotoxic agents. The result indicated that four newly synthesized compounds exhibited good cytotoxic activities. Especially, the most potent compounds **5a** and **5c** also showed higher cytotoxic activities against the K562 cell line, which is characterized by IC_{50} values of 56.91 μM and 58.55 μM. The lead compounds emerging with the most potent antitumor activity in this study (such as **5a** and **5c**) will be further structurally modified towards the discovery of a compound with optimal anticancer activities.

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